

C20 susceptibility testing. EHEC and non-EHEC Ecoli were picked from McConkey plates after testing for stxI or stxII or both toxin genes in colony hybridization and MIC testing was performed according to NCCLS guidelines for enterobacteria.

IN THE CLAIMS

1. (Amended). A Polymerase Chain Reaction (PCR) method for detection and differentiation of pathogenic enterobacteria in a sample, wherein a set of oligonucleotide primer pairs is added to said sample, each primer pair being capable of specifically amplifying a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of pathogenic *E. coli*, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for amplification of each subgroup at least one primer is added to the sample.

2. (Amended) The method according to claim 1 wherein the set of oligonucleotide primer pairs comprises primer pairs selected from

121 a primer pair that hybridizes to a gene encoding heat labile toxin, or heat stable toxin for amplification of a DNA sequence characteristic for enterotoxigenic *E. coli*;

a primer pair that hybridizes to a gene encoding heat stable toxin for amplification of a DNA sequence characteristic for enteroaggregative *E. coli*;

a primer pair that hybridizes to the pCVD432 plasmid for amplification of a DNA sequence characteristic for enteroaggregative *E. coli*;

a primer pair that hybridizes to the inv-plasmid for amplification a DNA sequence contained in enteroinvasive *E. coli*;

a primer pair that hybridizes to the EAF plasmid, or the eae gene for amplification of a DNA sequence characteristic for enteropathogenic *E. coli*;

a primer pair that hybridizes to the genes encoding shiga-like toxin stxI or stxII for amplification of a DNA sequence characteristic for enterohemorrhagic *E. coli*.

3. (Amended) The method according to claim 2 wherein

the primer pair that hybridises to the gene encoding heat labile toxin characteristic for enterotoxigenic *E. coli* is

LT-1: 5' GCG TTA CTA TCC TCT CTA TGT G 3' (SEQ ID NO.: 1) and
LT-2: 5' AGT TTT CCA TAC TGA TTG CCG C 3' (SEQ ID NO.: 2);

the primer pair that hybridises to the gene encoding heat stabile toxin characteristic for enterotoxigenic *E. coli* is

ST-1: 5' TCC CTC AGG ATG CTA AAC CAG 3' (SEQ ID NO.: 3) and
ST-2a: 5' TCG ATT TAT TCA ACA AAG CAA C 3' (SEQ ID NO.: 4);

the primer pair that hybridises for the gene encoding heat stabile toxin characteristic for enteroaggregative *E. coli* is

EASTI-1: 5' AAC TGC TGG GTA TGT GGC TGG 3' (SEQ ID NO.: 5) and
EASTI-2: 5' TGC TGA CCT GCC TCT TCC ATG 3' (SEQ ID NO.: 6);

the primer pair which hybridises to the pCVD432 plasmid is

EA-1: 5' CTG GCG AAA GAC TGT ATC ATT G 3' (SEQ ID NO.: 7) and
EA-2: 5' TAA TGT ATA GAA ATC CGC TGT T 3' (SEQ ID NO.: 8);

the primer pair which hybridises to the inv-plasmid is

EI-1: 5' TTT CTG GAT GGT ATG GTG AGG 3' (SEQ ID NO.: 9) and
EI-2: 5' CTT GAA CAT AAG GAA ATA AAC 3' (SEQ ID NO.: 10);

the primer pair which hybridises to the EAF plasmid is

EP-1: 5' CAG GGT AAA AGA AAG ATG ATA AG 3' (SEQ ID NO.: 11) and
EP-2: 5' AAT ATG GGG ACC ATG TAT TAT C 3' (SEQ ID NO.: 12);

the primer pair which hybridises to the eae gene is

EPeh-1: 5' CCC GCA CCC GGC ACA AGC ATA AG 3' (SEQ ID NO.: 13) and
EPeh-2: 5' AGT CTC GCC AGT ATT CGC CAC C 3' (SEQ ID NO.: 14);

the primer pair which hybridises to the gene encoding shiga-like toxin SttI is

SttI-1: 5' ATG AAA AAA ACA TTA TTA ATA GC 3' (SEQ ID NO.: 15) and
SttI-2: 5' TCA CYG AGC TAT TCT GAG TCA AGC 3' (SEQ ID NO.: 16);

the primer pair which hybridises to the gene encoding shiga-like toxin SttII is

SlitII-1: 5' ATG AAG AAG ATR WTT RTD GCR CYT TTA TTY G 3' (SEQ ID NO.: 17) and
SlitII-2: 5' TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC 3' (SEQ ID NO.: 18)

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

4. (Amended). The method according to claim 1 wherein a polymerase having additional 5'-3' exonuclease activity is used for the amplification of DNA, and an oligonucleotide probe labeled at the most 5' base with a fluorescent dye and at the most 3' base with a fluorescent quencher dye and wherein said probe hybridizes within the target DNA is included in the amplification process; said labeled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by said pol.

7. (Amended) The method according to claim 6 wherein the labelled oligonucleotide probe for the detection of heat labile toxin characteristic for enterotoxigenic *E. coli* is

5' AGC TCC CCA GTC TAT TAC AGA ACT ATG 3' (SEQ ID NO.: 19)

the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic forenterotoxigenic *E. coli* is

5' ACA TAC GTT ACA GAC ATA ATC AGA ATC AG 3' (SEQ ID NO.: 20);

the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic for enteroaggregative *E. coli* is

5' ATG AAG GGG CGA AGT TCT GGC TCA ATG TGC 3' (SEQ ID NO.: 21);

the labelled oligonucleotide probe for the detection of pCVD432 plasmid is

3' CTC TTT TAA CTT ATG ATA TGT AAT GTC TGG 3' (SEQ ID NO.: 22);

the labelled oligonucleotide probe for the detection of the inv-plasmid is

5' CAA AAA CAG AAG AAC CTA TGT CTA CCT 3' (SEQ ID NO.: 23)

the labelled oligonucleotide probe for the detection of the EAF-plasmid is

5' CTT GGA GTG ATC GAA CGG GAT CCA AAT 3' (SEQ ID NO.: 24);

the labelled oligonucleotide probe for the detection of the eae gene is

5' TAA ACG GGT ATT ATC AAC AGA AAA ATC C 3' (SEQ ID NO.: 25);

the labelled oligonucleotide probe for the detection of shiga-like toxin SlI gene is

5' TCG CTG AAT CCC CCT CCA TTA TGA CAG GCA 3' (SEQ ID NO.: 26);

the labelled oligonucleotide probe for the detection of shiga-like toxin SlII gene

is

5' CAG GTA CTG GAT TTG ATT GTG ACA GTC ATT 3' (SEQ ID NO.: 27).

10. (Amended) A set of oligonucleotide primer pairs useful for polymerase chain reaction (PCR) amplification of DNA of pathogenic enterobacteria allowing detection and differentiation of pathogenic enterobacteria in a sample, wherein each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of the pathogenic *E. coli* strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for amplification of each subgroup at least one primer is included in said set of oligonucleotide probes.

11. (Amended) The set of primer pairs according to claim 10 comprising

a primer pair that hybridizes to a gene encoding heat labile toxin, or heat stabile toxin of enterotoxigenic *E. coli*;

a primer pair that hybridizes to a gene encoding heat stabile toxin of enteroaggregative *E. coli*;

a primer pair that hybridizes to the pCVD432 plasmid of enteroaggregative *E. coli*;

a primer pair that hybridizes to the inv-plasmid of enteroinvasive *E. coli*;
a primer pair that hybridizes to the EAF plasmid, or the eae gene of enteropathogenic *E. coli*;

a primer pair that hybridizes to the gene encoding shiga-like toxin sltI or sltII of enterohemorrhagic *E. coli*.

12. (Amended) The set of primer pairs according to claim 11 wherein
the primer pair which hybridises to the gene encoding heat labile toxin of
enterotoxigenic *E. coli* is

LT-1: 5' GCG TTA CTA TCC TCT CTA TGT G 3' (SEQ ID NO.: 1) and
LT-2: 5' AGT TTT CCA TAC TGA TTG CCG C 3' (SEQ ID NO.: 2);

the primer pair which hybridises to the gene encoding heat stabile toxin of
enterotoxigenic *E. coli* is

ST-1: 5' TCC CTC AGG ATG CTA AAC CAG 3' (SEQ ID NO.: 3) and
ST-2a: 5' TCG ATT TAT TCA ACA AAG CAA C 3' (SEQ ID NO.: 4);

the primer pair which hybridises to the gene encoding heat stabile toxin of
enteroaggregative *E. coli* is

EASTI-1: 5' AAC TGC TGG GTA TGT GGC TGG 3' (SEQ ID NO.: 5) and
EASTI-2: 5' TGC TGA CCT GCC TCT TCC ATG 3' (SEQ ID NO.: 6);

the primer pair which hybridises to the pCVD432 plasmid is

EA-1: 5' CTG GCG AAA GAC TGT ATC ATT G 3' (SEQ ID NO.: 7) and
EA-2: 5' TAA TGT ATA GAA ATC CGC TGT T 3' (SEQ ID NO.: 8);

the primer pair which hybridises to the inv-plasmid is

EI-1: 5' TTT CTG GAT GGT ATG GTG AGG 3' (SEQ ID NO.: 9) and
EI-2: 5' CTT GAA CAT AAG GAA ATA AAC 3' (SEQ ID NO.: 10)

the primer pair which hybridises to the EAF plasmid is

EP-1: 5' CAG GGT AAA AGA AAG ATG ATA AG 3' (SEQ ID NO.: 11)
and
EP-2: 5' AAT ATG GGG ACC ATG TAT TAT C 3' (SEQ ID NO.: 12);

the primer pair which hybridises to the eae gene is

EPeh-1: 5' CCC GGA CCC GGC ACA AGC ATA AG 3' (SEQ ID NO.: 13)
and
EPeh-2: 5' AGT CTC GCC AGT ATT CGC CAC C 3' (SEQ ID NO.: 14);

the primer pair which hybridises to the shiga-like toxin stxI gene is

StxI-1: 5' ATG AAA AAA ACA TTA TTA ATA GC 3' (SEQ ID NO.: 15) and
StxI-2: 5' TCA CYG AGC TAT TCT GAG TCA AGC 3' (SEQ ID NO.: 16);

the primer pair which hybridises to the shiga-like toxin stII is

stII-1: 5' ATG AAG AAG ATR WTT RTD GCR GYT TTA TTY G^{3'} (SEQ ID NO.: 17) and
stII-2: 5' TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC^{3'} (SEQ ID NO.: 18)

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

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13. (Amended) A set of labeled oligonucleotide probes useful for detection and differentiation of pathogenic enterobacteria in a sample by Real Time-PCR, each probe specifically binding a sequence of a virulence factor/toxin genes characteristic of one of the subgroups of pathogenic *E. coli* strains comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for detection and differentiation of each subgroup at least one probe is included in set of oligonucleotide probes.

15. (Amended) The set of probes according to claim 14 wherein
the labelled oligonucleotide probe for the detection of heat labile toxin characteristic for enterotoxigenic *E. coli* is

5' AGC TCC CCA GTC TAT TAC AGA ACT ATG^{3'} (SEQ ID NO.: 19);

the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic for enterotoxigenic *E. coli* is

5' ACA TAC GTT ACA GAC ATA ATC AGA ATC AG^{3'} (SEQ ID NO.: 20);

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the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic for enteroaggregative *E. coli* is

5' ATG AAG GGG CGA AGT TCT GGC TCA ATG TGC^{3'} (SEQ ID NO.: 21);

the labelled oligonucleotide probe for the detection of pCVD432 plasmid is

5' CTC TTT TAA CTT ATG ATA TGT AAT GTC TGG^{3'} (SEQ ID NO.: 22);

the labelled oligonucleotide probe for the detection of the inv-plasmid is

5' CAA AAA CAG AAG AAC CTA TGT CTA CCT^{3'} (SEQ ID NO.: 23)

the labelled oligonucleotide probe for the detection of the EAF-plasmid is

5' CTT GGA GTG ATC GAA CGG GAT CCA AAT 3' (SEQ ID NO.: 24)

the labelled oligonucleotide probe for the detection of the eae gene is

5' TAA ACG GGT ATT ATC AAC AGA AAA ATC C 3' (SEQ ID NO.: 25)

the labelled oligonucleotide probe for the detection of shiga-like toxin SttI gene is

5' TCG CTG AAT CCC CCT CCA TTA TGA CAG GCA 3' (SEQ ID NO.: 26);

the labelled oligonucleotide probe for the detection of shiga-like toxin SttII gene

is

5' CAG GTA CTG GAT TTG ATT GTG ACA GTC ATT 3' (SEQ ID NO.: 27).

16. (Amended) A kit useful for diagnosing an enterobacteria infection in samples derived from a living animal body including a human, by Real Time PCR method, said kit comprising:

(a) a set of oligonucleotide primer pairs, wherein said primer pair allows detection and differentiation of pathogenic enterobacteria in a sample, wherein each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of the pathogenic *E. coli* strains said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for amplification of each subgroup at least one primer is included in said set of oligonucleotide probes and (b) a set of oligonucleotide probes, wherein said set of oligonucleotide probes detect virulence factor/toxin genes characteristic of one of the subgroups of pathogenic *E. coli* strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains by real time PCR.

REMARKS

Amendments to the Specification

The amendments to the specification are purely ministerial. The specification was objected to because the use of the trademark Taqman™ was not capitalized when recited or